Enumeration and Identity of *Campylobacter* spp. in Italian Broilers

G. Manfreda,*[1] A. De Cesare,* V. Bondioli,* N. J. Stern,† and A. Franchini*

*Department of Food Science, Alma Mater Studiorum—University of Bologna, Bologna, Italy; and †USDA-ARS, Athens, GA 30604

ABSTRACT Transmission of *Campylobacter* to humans has been prominently associated with mishandling or improperly preparing contaminated poultry carcasses. The number of organisms per carcass represents an important measure of human exposure to the agent. Therefore, we wished to estimate this public exposure over 1 yr among Italian broiler carcasses. We sampled 213 broiler carcasses from rinse water samples collected from a single slaughterhouse. Groups of carcasses had mean processed weights ranging from 1.2 to 2.7 kg. These were produced from 22 commercial broiler chicken flocks collected from 12 different farms, 3 of which were seasonally tested. Carcasses were rinsed with sterile water, and the rinse suspension was then serially diluted and spread-plated directly onto Campy-Cefex agar plates. One to 5 typical *Campylobacter* colonies per plate were identified by polymerase chain reaction as *Campylobacter* thermo-tolerant species. The overall estimated mean count per carcass in our study was 5.16 ± 0.80 $\log_{10}$ cfu. This value increased in summer and autumn, as well as on carcasses collected from farms located >100 km far from the slaughterhouse. A total of 678 *Campylobacter* colonies were identified by polymerase chain reaction. The majority of isolates were classified as *Campylobacter jejuni* (49.2%) or *Campylobacter coli* (47.5%). The overall number of *C. jejuni* was significantly higher on 1) carcasses weighing >2 kg, 2) carcasses belonging to flocks with >10,000 birds, and 3) carcasses collected from farms located >100 km from the slaughterhouse. Moreover, among farms tested seasonally, *C. jejuni* was significantly greater than *C. coli* in winter. These data provide the first results of a continuing survey on *Campylobacter* loads and species identification from Italian broiler carcasses and represents an important baseline to estimate the human exposure to *Campylobacter* in Italy.

Key words: *Campylobacter*, enumeration, broiler, rinse water, thermophilic species

2006 Poultry Science 85:556–562

INTRODUCTION

Researchers have long suggested that a substantial portion of human campylobacteriosis is associated with poultry (Norkrans and Svedhem, 1982; Hopkins and Scott, 1983; Oosterom et al., 1984; Harris et al., 1986; Deming et al., 1987; Kapperud et al., 1992; Tauxe, 1992; Frost, 2001; Stern et al., 2003). *Campylobacter* can be found in broilers on the farm, during processing, and at retail markets (Berrang et al., 2004). Intestinal contents and feces of broilers can harbor *Campylobacter* spp. (Jeffrey et al., 2001). During the course of slaughter and processing, there is potential for the alimentary tract (from the crop to the colon) to leak or rupture, spilling contents onto the skin or muscle of broiler carcasses. Once on the surface of a carcass, such contamination has the potential to persist through the remainder of the carcass processing (Byrd et al., 2002) and potentially to cross-contaminate carcasses belonging to different flocks (Newell et al., 2001).

Human infections with *Campylobacter* spp. (campylobacteriosis) are predominantly caused by the members of the thermotolerant group including *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* (Griffiths and Park, 1990). *Campylobacter jejuni* represents the species most frequently isolated in cases of human gastroenteritis in developed countries. In The Netherlands, England, and Wales, as well as in the United States, campylobacteriosis varies from 6.8 to 8.3 to 9.4 cases/1,000 person-yr, respectively (Schouls et al., 2003). The true frequency of disease depends on the methods used for detection as well as the fidelity given to reporting these cases. Optimum methods and reporting are still being discussed.

In Italy, incomplete data are currently available for human campylobacteriosis and incidence of *Campylobacter* in broilers and poultry meat. Pezzotti et al. (2003) investigated the prevalence of *Campylobacter* colonization in broilers at the time of slaughter and the contamination rate of raw poultry meat samples at processing plants or at retail markets in northeastern Italy during 2000 and 2001. The infection rate among broilers was 82.9%; 8.2% of the flocks were contaminated with multiple *Campylobacter* species. Among the reported isolates, 44.4 and 55.6% of these were identified as *C. jejuni* and *C. coli*, respectively. The contamination frequency in chicken meat was 81.3%.

Received June 9, 2005.
Accepted October 24, 2005.
[1]Corresponding author: gmanfreda@disa.unibo.it

Moreover, 56.2 and 43.8% of the chicken isolates were identified as *C. jejuni* and *C. coli*, respectively.

As concern over this organism has increased relative to food safety, so has the need for efficient, effective methods for its detection and enumeration in poultry products. There are a number of approaches that can be taken to detect *Campylobacter* from poultry products. These approaches include direct plating and enrichment of neck skin, swabs, rinses, and even incubation of the entire product in enrichment broth (Musgrove et al., 2003). However, enumeration is crucial because *Campylobacter* levels found on carcasses may represent an important source of consumer exposure and a potential risk for infection (Stern et al., 2003). Direct plating of carcass rinses is often used by regulatory and research agencies (Ransom and Rose, 1998; Stern and Robach, 2003). Semiquantitative protocols (Josefsen et al., 2004) and enrichment coupled with most probably number procedures are also available. These last methods are more expensive, time-consuming, susceptible to far more technician error, and do not necessarily result in improved estimation of levels of *Campylobacter* spp. as compared with direct plating.

The goals of the present study were 1) to quantify the *Campylobacter* contamination levels in Italian poultry operations collected from different farms and processed in the same slaughterhouse, 2) to test the influence of different parameters (i.e., season, distance from farm to slaughterhouse, numbers of birds per flock, and processed carcass weight) on carcass *Campylobacter* loads, 3) to evaluate the incidence of various thermo-tolerant *Campylobacter* species among the isolates collected from fully processed broiler carcasses, and 4) to estimate the factors that might influence the species detected on carcasses.

MATERIALS AND METHODS

Broiler Chicken Carcasses

Two hundred thirteen broiler carcasses were collected in one Italian slaughterhouse between October 14, 2003 and September 2, 2004 (Table 1). All carcasses were obtained from commercial broiler chickens among 22 flocks reared on 12 different farms labeled A to N (Table 1). Farms B, F, and M were located >100 km far from the slaughterhouse, whereas other farms were nearer. Flocks reared on farms A, B, and C were tested repeatedly for *Campylobacter* seasonal trends. Based on varying anticipated temperatures during each sampling, October and November were designated as autumn, December through March as winter, April and May as spring, and June through September as summer.

During each sample date, 18 to 20 fully processed carcasses were removed after the air-cooling operation. Eight to 10 carcasses, processed as the first group in the sampling day, belonged to farms A, B, or C and had a mean weight ranging from 2.1 to 2.7 kg, corresponding to an average age of 56 d. Nine to 10 carcasses, processed as second to the fifth groups of the day, belonged to one of the other farms and had a mean weight ranging from 1.2 to 1.9 kg, corresponding to an average age of 46 d. The carcass weights were evaluated as higher or lower than 2 kg.

The number of birds per flock, evaluated as greater or fewer than 10,000 birds, is described in Table 1.

Sampling Procedure

All carcasses were aseptically placed in sterile plastic bags and rinsed with different volumes of sterile water according to their weight. Carcasses with weights ranging from 2.1 to 2.7 kg were rinsed with 300 mL of sterile water; carcasses ranging from 1.2 to 1.9 kg were rinsed in 200 mL of sterile water. Bags were vigorously shaken by hand for approximately 1 min. After sampling was complete, carcasses were removed using sterile gloved hands, and the rinses were aseptically transferred to smaller sterile bags chilled at 4°C, which were then transferred to the laboratory within 3 h.

Detection and Enumeration of Campylobacter

Campylobacter was quantified as previously described (Stern and Line, 1992). Briefly, 0.1 mL of 3 rinse aliquots of serial dilutions was spread-plated onto duplicate Campy-Cefex agar plates (Stern and Line, 1992) for quantitative analysis. The inoculated plates were incubated at 42°C for 24 to 48 h in a microaerobic atmosphere (85% N_2, 10% CO_2, 5% O_2), obtained by flushing the gas mixture through plastic zip-locking freezer bags that were then hermetically sealed. After incubation, characteristic *Campylobacter* colonies were presumptively identified through phase-contrast microscopical observation for typical morphological aspects and corkscrew movement. Then, the colonies were enumerated, and dilution was considered to calculate estimated counts per carcass.

Identification of Thermo-tolerant Campylobacter

One to 5 typical *Campylobacter* colonies isolated from broiler chicken rinses among 16 flocks were collected from 8 of the 12 farms tested (i.e., farm A, flocks 1, 7, and 13; farm B, flocks 3, 9, 15, and 19; farm C, flocks 5, 11, 17, and 21; farm D, flock 2; farm E, flock 4; farm F, flock 6; farm G, flock 8; and farm H, flock 10) and purified through 3 subcultures on blood agar plates (Oxoid, Garbagnate M.se, Milano, Italy) supplemented with 5% lysed horse blood (Oxoid) and incubated as previously described.

Chromosomal DNA from the *Campylobacter* purified colonies and from positive control strains was extracted from 24-h blood agar plate cultures suspended in 1.5 mL of sterile water and centrifuged for 5 min at 18,000 × *g* at 4°C. The pellet was resuspended in 300 μL of 6% Chelex 100 resin suspension (BioRad, Segrate, Milano, Italy) and incubated for 20 min in a 56°C water bath (Josefsen et al., 2004). Following incubation, each suspension was vor-

Table 1. *Campylobacter* mean loads on broiler carcasses collected from different flocks

Flock identity	Farm identity (birds, n)	Birds per flock (n)	Sampling date	*Campylobacter* count per carcass (log_{10} cfu[1])
1	Farm A (9)	15,340	October 14, 2003	5.42 ± 0.84[ab]
2	Farm D (10)	4,029	October 14, 2003	5.05 ± 0.38[ab]
3	Farm B (10)	3,100	November 11, 2003	6.07 ± 0.47[b]
4	Farm E (10)	8,928	November 11, 2003	6.09 ± 0.37[b]
5	Farm C (8)	13,360	December 2, 2003	4.74 ± 0.38[ab]
6	Farm F (10)	8,343	December 2, 2003	4.91 ± 0.50[ab]
7	Farm A (10)	17,280	January 14, 2004	4.23 ± 0.87[a]
8	Farm G (10)	5,376	January 14, 2004	5.40 ± 0.93[ab]
9	Farm B (9)	11,040	February 17, 2004	4.93 ± 0.40[ab]
10	Farm H (9)	6,912	February 17, 2004	4.66 ± 0.29[ab]
11	Farm C (10)	13,360	March 11, 2004	5.41 ± 0.54[ab]
12	Farm I (10)	8,160	March 11, 2004	5.50 ± 0.62[ab]
13	Farm A (10)	7,680	April 8, 2004	5.29 ± 0.93[ab]
14	Farm D (10)	8,928	April 8, 2004	3.93 ± 0.38[a]
15	Farm B (9)	10,591	May 11, 2004	5.20 ± 0.43[ab]
16	Farm L (9)	9,385	May 11, 2004	4.55 ± 0.71[ab]
17	Farm C (10)	15,360	June 9, 2004	6.13 ± 1.06[b]
18	Farm M (10)	6,080	June 9, 2004	5.31 ± 0.63[ab]
19	Farm B (10)	10,816	August 4, 2004	5.48 ± 0.78[ab]
20	Farm G (10)	8,000	August 4, 2004	4.97 ± 0.33[ab]
21	Farm C (10)	12,000	September 2, 2004	4.89 ± 0.29[ab]
22	Farm N (10)	15,300	September 2, 2004	5.32 ± 0.44[ab]

[a,b]Numbers without common superscripts differ significantly ($P \leq 0.05$).

[1]Means ± SD.

texed for 10 s and incubated in a 100°C water bath for 8 min followed by immediate chilling on ice. Then, it was centrifuged for 5 min at 18,000 × *g* at 4°C, and 5 μL of the supernatant was used as a template in the PCR assays.

The species *C. jejuni* and *C. coli* were identified using the multiplex PCR published by Manfreda et al. (2003). The amplification reactions were achieved using the following program: 1 cycle of 10 min at 95°C; 35 cycles of 30 s each at 95°C, 1.30 min at 59°C, 1 min at 72°C; and 1 cycle of 10 min at 72°C. Following the amplification, 20 μL of the PCR products was electrophoresed in 1× Tris-acetate-EDTA (Roche Diagnostics, Mannheim, Germany) on 1.5% (wt/vol) Seakem LE Agarose (FMC Bioproducts, Rockland, ME) stained with ethidium bromide (1 μg/mL) and visualized under ultraviolet light. The expected PCR amplicons were at 857, 589, and 462 bp, corresponding to the genus *Campylobacter*, *C. jejuni*, and *C. coli*, respectively.

The specie *Campylobacter lari* was identified using the PCR protocol published by Linton et al. (1996). The amplification reactions were performed according to the following program: 1 cycle of 10 min at 94°C; 24 cycles of 60 s each at 94°C, 60 s at 64°C, 60 s at 72°C; and 1 cycle of 10 min at 72°C. Following the amplification, 20 μL of the PCR products was electrophoresed and visualized as previoulsy described. The expected PCR amplicon was at 561 bp.

All amplification reactions were achieved in the Master Cycler Gradient (Eppendorf, Milano, Italy). The positive control strains were represented by *C. jejuni* RM 1221, *C. coli* RM 2228, and *C. lari* CCUG 22395, whereas *Staphylococcus aureus* ATCC 51740 was used as a negative control.

Statistical Analysis

The data collected were analyzed with the Statgraphics package (version 5.1, StatSoft, Inc., Padova, Italy). Arithmetic counts of cfu per carcass were transformed into log_{10} values and analyzed with the ANOVA procedure. The mean *Campylobacter* counts per carcass and 95% confidence intervals in each flock were calculated across each replication of 8 to 10 carcasses (Table 1).

Campylobacter mean counts per carcass between flocks were compared via the Scheffè test, and $P \leq 0.05$ was considered statistically significant (Table 1).

The effects of distance from farm to slaughterhouse and season (Table 2) as well as distance from farm to slaughterhouse and carcass weight (Table 3) on the *Campylobacter* mean counts per carcass were evaluated using the following model:

Table 2. Influence of distance from farm to slaughterhouse and season on the *Campylobacter* mean load per carcass

Distance	Season (samples, n)	Log_{10} cfu per carcass[1]
<100 km	Autumn (29)	5.52 ± 0.69[bc]
<100 km	Winter (57)	5.00 ± 0.79[ab]
<100 km	Spring (29)	4.59 ± 0.89[a]
<100 km	Summer (30)	5.33 ± 0.86[bc]
>100 km	Autumn (10)	6.07 ± 0.46[c]
>100 km	Winter (19)	4.91 ± 0.44[ab]
>100 km	Spring (9)	5.20 ± 0.42[abc]
>100 km	Summer (30)	5.36 ± 0.61[bc]

[a–c]Numbers without common superscripts differ significantly ($P \leq 0.05$).

[1]Means ± SD.

Table 3. Influence of distance from farm to slaughterhouse and carcass weight on the *Campylobacter* mean load per carcass

Distance	Carcass weight (samples, n)	Log_{10} cfu per carcass[1]
<100 km	<2 kg (78)	5.01 ± 0.81[a]
<100 km	>2 kg (67)	5.16 ± 0.92[ab]
>100 km	<2 kg (30)	5.18 ± 0.54[ab]
>100 km	>2 kg (38)	5.42 ± 0.67[b]

[a,b]Numbers without common superscripts differ significantly ($P \leq 0.05$).

[1]Means ± SD.

$$Y_{ijk} = \mu + D_i + S_j + DS_{ij} + e_{ijk}$$

where Y_{ijk} = value of the first carcass load, μ = population mean, D_i = effect of distance I from farm to slaughterhouse (i = greater than and less than 100 km), S_j = effect of season j (j = autumn, winter, spring, and summer), and e_{ijk} = residual error.

The distribution of the thermophilic species identified among the poultry rinse isolates in relation to the distance from farm to slaughterhouse, number of birds per flock, and processed carcass weights was evaluated using the χ^2 statistical test (Table 4).

The same analysis was performed to evaluate the distribution of the *Campylobacter* species among the birds reared in the farms seasonally tested (i.e., farms A, B, and C; Table 5).

RESULTS

Enumeration of Campylobacter in Broiler Chicken Rinses

Each of the 213 fully processed broiler carcasses sampled were *Campylobacter* positive. The overall estimated mean count per carcass was 5.16 ± 0.80 log_{10} cfu. In particular, the estimated count per carcasses with weight ranges of 2.1 to 2.7 kg varied from 3.18 to 7.99 log_{10} cfu. The mean value was 5.26 ± 0.85 log_{10} cfu. The count per carcass on broilers with weight ranges of 1.2 to 1.9 kg varied from 3.31 to 7.81 log_{10} cfu; the mean value was 5.06 ± 0.74 log_{10} cfu.

Table 1 summarizes the *Campylobacter* mean values per carcass in the 22 broiler chicken flocks examined. These mean values spanned from 3.93 (flock 14) to 6.13 log_{10} cfu (flock 17). The *Campylobacter* mean values detected for flocks 7 and 14 were significantly lower ($P < 0.05$) than those detected for flocks 3, 4, and 17 (Table 1). Statistical variations were observed in the *Campylobacter* count per carcass detected on birds belonging to different flocks reared in the same farm (i.e., flock 7 vs. 1 and 13 for farm A; flock 3 vs. 9, 15, and 19 for farm B; flock 17 vs. 5, 11, and 21 for farm C).

The overall *Campylobacter* mean counts per carcass detected on broilers reared in farms located closer (n = 145) or further (n = 68) than 100 km from the slaughterhouse were significantly different (5.09 vs. 5.32 log_{10} cfu per carcass). In contrast, the *Campylobacter* mean counts per carcass detected on broilers reared in flocks of greater (n = 95) or fewer (n = 118) than 10,000 birds did not represent a statistically ($P < 0.05$) significant difference (5.18 vs. 5.15 log_{10} cfu). In the same way, any statistically significant difference was detected among broilers with weights greater than (n = 105) or less than (n = 108) 2 kg (5.26 vs. 5.06). Finally, the *Campylobacter* mean values per carcass detected in autumn and summer (i.e., 5.66 and 5.34 log_{10} cfu) were significantly greater than those detected in winter and spring (i.e., 4.98 and 4.73 log_{10} cfu).

Both distance from farm to slaughterhouse and sampling season influenced the *Campylobacter* mean load per carcass. In fact, the mean values per carcass detected on animals processed in spring and reared on farms located <100 km far from the slaughterhouse were significantly lower ($P < 0.05$) than those found on animals processed in autumn and summer and reared on farms located >100 km far from the slaughterhouse (Table 2).

Table 3 shows the influence of both distance from farm to slaughterhouse and mean bird processed weight on the *Campylobacter* mean values per carcass. These last values detected on animals with weight <2 kg and reared on farms located <100 km from the slaughterhouse were significantly lower ($P < 0.05$) than those detected on processed carcasses with a mean weight >2 kg and reared on farms located >100 km from the slaughterhouse (5.01 vs. 5.42 log_{10} cfu per carcass).

Identification of Thermo-tolerant Campylobacter from Broiler Chicken Rinse

A total of 678 *Campylobacter* colonies were tested by PCR (Table 6). In particular, 474 colonies were isolated

Table 4. Distribution of *Campylobacter jejuni* and *Campylobacter coli* among the isolates tested in relation to different parameters

Parameters analyzed	Percentage of *C. jejuni*	Percentage of *C. coli*	*P*[1]
Distance from farm to slaughterhouse >100 km	62.14	37.86	≤0.0001
Distance from farm to slaughterhouse <100 km	45.78	54.22	
Birds per flock >10,000	68.98	31.02	<0.0001
Birds per flock <10,000	22.13	77.87	
Bird weight >2.0 kg	60.65	39.44	<0.0001
Bird weight <2.0 kg	27.60	72.40	

[1]*P* = Probability of χ^2 value.

Table 5. Distribution of *Campylobacter jejuni* and *Campylobacter coli* among the carcass rinse isolates collected from birds reared on farms A, B, and C, which were seasonally tested

	Autumn	Winter	Spring	Summer	P^1
Percentage of *C. jejuni*	46.30	75.16	50.51	56.67	≤0.0002
Percentage of *C. coli*	53.70	24.84	49.49	43.33	

[1]P = Probability of χ^2 value.

from animals reared on farms A, B, and C, and 204 colonies were isolated from birds belonging to flocks 2, 4, 6, 8, and 10 (Table 6).

Three hundred thirty-four colonies (i.e., 49.2%) were identified as *C. jejuni*, and 322 (47.5%) were identified as *C. coli*. Moreover, 3 colonies were mixed cultures of *C. jejuni* and *C. coli*, likely because of inadequate isolate separation; 19 isolates were positive for a member of *Campylobacter* genus but were not further identified. No colonies were identified as *C. lari*. *Campylobacter jejuni* was the prevalent species among isolates from flocks 2, 9, 11, and 15, whereas *C. coli* was the prevalent species among isolates from flocks 6, 7, 8, 10, and 13 (Table 6).

In comparison with the percentage of *C. coli*, the percentage of isolates identified as *C. jejuni* was significantly higher on birds reared on farms located >100 km from the slaughterhouse (i.e., 37.86 vs. 62.14), on birds collected from flocks containing >10,000 animals (31.02 vs. 68.98), and on birds with a mean weight >2 kg (39.44 vs. 60.56; Table 4). Moreover, the percentages of strains identified as *C. jejuni* during winter and summer on broilers reared on farms A, B, and C, which were seasonally tested, were significantly higher than those of strains identified as *C. coli*. Otherwise, during autumn and spring, any statistically significant differences were detected between the number of isolates classified in the 2 species (Table 5).

DISCUSSION

According to Stern and Robach (2003), *Campylobacter* levels found on carcasses may represent an important source, providing consumer exposure and a potential risk for infection. As *C. jejuni* and *C. coli* are only capable of growth between 30 and 46°C and require microaerobic conditions, it is highly unlikely that levels of the organism will increase on contaminated carcasses. The levels on the processed carcasses represent a true consumer risk for infection, as only 500 cells have been shown to cause human disease. Although cooking and proper handling of poultry products eliminate all risks, people continue to make mistakes, resulting in disease. Consequently, a reduction in levels of public exposure associated with carcasses would reasonably reduce disease. This appears to have been the case in Iceland, where interventions have reduced public exposure through poultry with the concomitant reduction in human disease (Stern et al., 2003).

There are a number of approaches that can be taken to detect *Campylobacter* from poultry products but, direct plating of carcass rinses is most often used by regulatory agencies (Ransom and Rose, 1998; Stern and Robach, 2003). The data collected in this work suggest that the mean load per Italian carcasses is approximately 5.16 $\log_{10}$ cfu with statistically significant differences among flocks reared in the same farm. The level of carcass contamination apparently increased in birds belonging to flocks reared on farms located >100 km far from the slaughterhouse where the birds were processed, whereas the level of contamination did not appear to be influenced by the number of birds per flock or bird processing weight. Stern et al. (1995) showed that *Campylobacter* numbers on both unprocessed carcass rinse samples and gut contents increase after catching and transport. Broilers defecate upon one another in the transport coups, and levels of *Campylo-*

Table 6. *Campylobacter* species identified among the poultry rinse isolates

Flock identity	Farm identity	Isolates tested, n	*Campylobacter jejuni*, n (%)	*Campylobacter coli*, n (%)
1	Farm A	48	22 (45.8)	20 (41.7)
2	Farm D	49	45 (91.8)	1 (2)
3	Farm B	15	3 (20)	9 (60)
4	Farm E	16	2 (12.5)	5 (31.2)
5	Farm C	29	18 (62.1)	11 (37.9)
6	Farm F	44	1 (2.3)	43 (97.7)
7	Farm A	33	6 (18.2)	26 (78.8)
8	Farm G	50	2 (4)	48 (96)
9	Farm B	50	49 (98)	1 (2)
10	Farm H	45	3 (6.6)	42 (93.3)
11	Farm C	50	48 (96)	2 (4)
13	Farm A	49	0	49 (100)
15	Farm B	50	50 (100)	0
17	Farm C	50	33 (66)	17 (34)
19	Farm B	50	25 (50)	25 (50)
21	Farm C	50	27 (54)	23 (46)
Total		678	334 (49.2)	322 (47.5)

bacter contamination on carcasses increase. Faecal shedding of *Campylobacter* increase, and holding the birds at the processing plant prior of slaughter does not reduce levels (Whyte et al., 2001).

In the present study, the overall *Campylobacter* mean value per carcass detected in summer and autumn was significantly higher that that detected in winter and spring, even if the differences were <1 log. According to other researchers, seasonal variation in *Campylobacter* contamination levels demonstrates the importance of conducting long-term surveys on chicken flocks rather than short-term surveys, which may give erroneous results that are dependent on the particular single season sampled (Meldrum et al., 2004).

We did not find evidence to support that processing plant cross-contamination played an important role contributing to increasing levels through the processing day. In fact, the levels per carcass among the first flocks sampled each day (i.e., those from farms A, B, and C) were, on average, higher than the counts observed later in the processing day (5.26 vs. 5.06 $\log_{10}$ cfu). Thus, interventions on the farm may provide greater probabilities for the control of *Campylobacter* spp. If high intestinal levels came into the plant in a particular flock, the numbers on the carcasses were higher. Previous flock contamination did not have as great an influence as the levels associated with each individual flock.

The majority (i.e., 96.7%) of isolates collected from carcass rinse water samples were identified as *C. jejuni* and *C. coli*. These versatile and metabolically active thermophilic *Campylobacter* species have a complete citric acid cycle and a complex and highly branched respiratory chain, which allows for both aerobic and anaerobic respiration (Kelly, 2001), as is present within the intestinal lumen. These properties enable *Campylobacter* to survive in a number of environments, in addition to the mammalian or avian gut, and may explain why *Campylobacter* infection has overtaken *Salmonella* as the leading cause of foodborne illness (Centers for Disease Control, 2004), even though *Campylobacter* species are generally more sensitive to environmental factors than are *Salmonella*. The results of a 1-yr surveillance study conducted in Italy in 2000 showed that *C. jejuni* and *C. coli* caused 82.7 and 17.3% of human food-borne infections (Carattoli et al., 2002).

In comparison with *C. coli*, *C. jejuni* was the prominent species on broiler carcasses weighing >2 kg, belonging to flocks with >10,000 birds, collected from farms located >100 km from the slaughterhouse (Table 4). Moreover, *c. jejuni* was more frequent than *C. coli* among the isolates collected during winter and summer (Table 5). These data seem to suggest that stress conditions such as extended transportation times, bird density, and age as well as extreme temperatures might select the *C. jejuni* isolates among the *Campylobacter* population contaminating broiler carcasses.

Thus far, in Italy, no systematic sampling procedure for continued evaluation of prevalence and levels of carcass contamination relating to the epidemiology of *Campylobacter* in poultry has been enacted. However, if poultry is considered as the major source for human campylobacteriosis, data on *Campylobacter* counts and species identity, as collected in this work, represent the first step to evaluate the efficacy of *Campylobacter* reduction measures that might be applied both on farms as well as in the slaughterhouses in the future to reduce the human exposure to this pathogen.

ACKNOWLEDGMENTS

The authors thank Pietro Olivi for his technical assistance.

REFERENCES

Berrang, M. E., D. P. Smith, W. R. Windham, and P. W. Feldner. 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* count. J. Food Prot. 67:235–238.

Byrd, J. A., B. M. Hargis, D. E. Corrier, R. L. Brewer, D. J. Caldwell, R. H. Bailey, J. L. McReynolds, K. L. Herron, and L. H. Stanker. 2002. Fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants. Poult. Sci. 81:70–74.

Carattoli, A., A. M. Dionisi, and I. Luzzi. 2002. Use of a LightCycler gyrA mutation assay for identification of ciprofloxacin-resistant *Campylobacter coli*. FEMS Microbiol. Lett. 214:87–93.

Centers for Disease Control. 2004. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-selected sites. United States. 2003. Morb. Mortal. Wkly. Rep. 53:338–343.

Deming, M. S., R. V. Tauxe, P. A. Blake, S. E. Dixon, B. S. Fowler, T. S. Jones, E. A. Lockamy, C. M. Patton, and R. O. Sikes. 1987. *Campylobacter* enteritis at a university from eating chickens and from cats. Am. J. Epidemiol. 126:526–534.

Frost, J. A. 2001. Current epidemiological issues in human campylobacteriosis. J. Appl. Microbiol. 90:85S–95S.

Griffiths, P. L., and R. W. A. Park. 1990. Campylobacters associated with human diarrhoeal disease. J. Appl. Bacteriol. 69:281–301.

Harris, N. V., N. S. Weiss, and C. M. Nolan. 1986. The role of poultry and meats in the etiology of *Campylobacter jejuni/coli* enteritis. Am. J. Publ. Hlth. 76:407–411.

Hopkins, R. S., and A. S. Scott. 1983. Handling raw chicken as a source for sporadic *Campylobacter jejuni* infections. J. Infect. Dis. 148:770.

Jeffrey, J. S., K. H. Tonooka, and J. Lozano. 2001. Prevalence of *Campylobacter* spp. from skin, crop, and intestine of commercial broiler chicken carcasses at processing. Poult. Sci. 80:1390–1392.

Josefsen, M. H., P. S. Lübeck, F. Hansen, and J. Hoorfar. 2004. Towards an international standard for PCR-based detection of foodborne thermotolerant campylobacters: Interaction of enrichment media and pre-PCR treatment on carcass rinse samples. J. Microbiol. Methods 58:39–48.

Kapperud, G., E. Skjerve, N. H. Bean, S. M. Ostroff, and J. Lassen. 1992. Risk factors for sporadic *Campylobacter* infections: Results of a case-control study in southeastern Norway. J. Clin. Microbiol. 30:3117–3121.

Kelly, D. J. 2001. The physiology and metabolism of *Campylobacter jejuni* and *Helicobacter pylori*. J. Appl. Microbiol. 90:16S–24S.

Linton, D., R. J. Owen, and J. Stanley. 1996. Rapid identification by PCR of the genus *Campylobacter*, and of five *Campylobacter* species enterophatogenic for man and animals. Res. Microbiol. 147:707–718.

Manfreda, G., A. De Cesare, V. Bondioli, and A. Franchini. 2003. Comparison of the BAX® System with multiplex PCR method for simultaneous detection and identification of *Campylo-*

bacter jejuni and *Campylobacter coli* in environmental samples. Int. J. Food Microbiol. 87:271–278.

Meldrum, R. J., D. Tucker, and C. Edwards. 2004. Baseline rates of *Campylobacter* and Salmonella in raw chickens in Wales, United Kingdom, in 2002. J. Food Prot. 67:1226–1228.

Musgrove, M. T., N. A. Cox, M. E. Berrang, and M. A. Harrison. 2003. Comparison of weep and carcass rinses for recovery of *Campylobacter* from retail broiler carcasses. J. Food Prot. 66:1720–1723.

Newell, D. G., J. E. Shreeve, M. Toszeghy, G. Dominigue, S. Bull, T. Humphrey, and G. Mead. 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. Appl. Environ. Microbiol. 67:2636–2640.

Norkrans, G., and Å. Svedhem. 1982. Epidemiologic aspects of *Campylobacter jejuni* enteritis. J. Hyg. Camb. 89:163–170.

Oosterom, J., C. H. den Uyl, J. R. J. Bänffer, and J. Huisman. 1984. Epidemiologic investigations on *Campylobacter jejuni* in households with primary infection. J. Hyg. Camb. 92:325–332.

Pezzotti, G., A. Serafin, I. Luzzi, R. Mioni, M. Milan, and R. Perin. 2003. Occurrence and resistance of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. Int. J. Food Microbiol. 82:271–287.

Ransom, G. M., and B. E. Rose. 1998. Isolation, identification and enumeration of *Campylobacter jejuni/coli* from meat and poultry products. Pages 6.1–6.10 in USDA/FSIS Microbiology Laboratory Guidebook. 3rd ed. B. B. Dey and C. P. Lattuada, ed. USDA, Washington, DC.

Schouls, L. M., S. Reulen, B. Duim, J. A. Wagenaar, R. J. L. Willems, K. E. Dingle, F. M. Colles, and J. D. A. Van Embden. 2003. Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: Strain diversity, host range, and recombination. J. Clin. Microbiol. 41:15–26.

Stern, N. J., M. R. Clavero, J. S. Bailey, N. A. Cox, and M. C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. Poult. Sci. 74:937–941.

Stern, N. J., K. L. Hiett, G. A. Alfredsson, K. G. Kristinsson, J. Reiersen, H. Hardardottir, H. Briem, E. Gunnarsson, F. Georgsson, R. Lowman, E. Berndtson, A. M. Lammerding, G. M. Paoli, and M. T. Musgrove. 2003. *Campylobacter* spp. in Iceland poultry operations and human disease. Epidemiol. Infect. 130:23–32.

Stern, N. J., and J. E. Line. 1992. Comparison of three methods for recovery of *Campylobacter* spp. from broiler carcasses. J. Food Prot. 55:663–666.

Stern, N. J., and M. C. Robach. 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. J. Food Prot. 66:1557–1563.

Tauxe, R. V. 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. Pages 9–19 in *Campylobacter jejuni*: Current Status and Future Trends. I. Nachamkin, M. Blaser, and L. S. Tompkins, ed. Am. Soc. Microbiol. Washington, DC.

Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony. 2001. The effect of transportation stress on excretion rates of Campylobacters in market-age broilers. Poult. Sci. 80:817–820.